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Title: "Transplacental Transmission of the COVID-19 Vaccine mRNA: Evidence from Placental, Maternal and Cord Blood Analyses Post-Vaccination"

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Keywords: COVID-19, Vaccine mRNA, Biodistribution, Placenta, neonate, pregnancy

Objective:

SARS-CoV-2 infection presents substantial challenges to global health, necessitating effective interventions such as COVID-19 vaccination. The initial clinical trials for the COVID-19 mRNA vaccines excluded pregnant women, leading to a knowledge gap concerning the potential biodistribution of the vaccine's mRNA to the placenta and or the fetus after maternal vaccination. The Pfizer and Moderna Assessment Reports provided to the European Medicines Agency[1,2] concluded that in animal models, a fraction of the administered mRNA dose is distributed to distant tissues, mainly the liver, adrenal glands, spleen, and ovaries. Another animal study showed that lipid nanoparticles (LNPs)-mRNA injections, similar in composition to COVID-19 mRNA vaccines, delivered functional mRNA to the placenta and other fetal organs.[3] Our recently published study demonstrated that the COVID-19 vaccine mRNA administered to lactating mothers can spread systemically from the injection site to breast milk, indicating it could cross the blood-milk barrier.[4,5] Another study evaluating the effects of maternal COVID-19 vaccination on the hematopoietic stem progenitor cells in the umbilical cord blood suggested that the LNPs/mRNA vaccines might reach the fetus following maternal vaccination.[6] This report presents two unique cases wherein pregnant individuals were vaccinated with the COVID-19 mRNA vaccine shortly before delivery. This study aimed to assess the presence of COVID-19 vaccine mRNA in the placenta and cord blood following maternal vaccination during human pregnancy.

Study Design:

This study involved two pregnant individuals. Patient #1, a 34-year-old gravida at 38 weeks and 4 days of gestation had pregnancy-induced hypertension and was vaccinated with two Pfizer COVID-19 vaccine doses and two booster doses (Pfizer and Moderna). The last dose was a Moderna booster administered two days before cesarean section delivery of a healthy baby. Samples from the placenta, maternal blood, and cord blood were collected post-delivery. Patient #2, a 33-year-old gravida at 40 weeks of gestation, had an uncomplicated pregnancy and received

two Pfizer COVID-19 vaccine doses; the last dose was administered 10 days before vaginal delivery of a healthy baby. Only placental samples were collected after birth.

COVID-19 vaccine mRNA was assayed by Droplet Digital PCR (ddPCR) in the placenta, cord, and maternal blood. Based on the putative sequences of the mRNA1273 (Moderna) and BNT162b2 (Pfizer) vaccines, two PCR assays targeting two regions of the vaccine mRNA were designed.[5] The vaccine mRNA localization in the placental sections was done by in situ hybridization (ISH) using RNAscope targeting the BNT162b2 and mRNA1273 vaccine sequences. Placental samples from mothers without COVID-19 (confirmed by PCR) and with no history of vaccination were used as the negative controls. We used placenta explants spiked with diluted BNT162b2 or mRNA1273 for positive controls. Placental expression of spike protein was evaluated using an automated capillary western blot system (WES). The stability of vaccine mRNA can be variable and may degrade during distribution and cellular entry. Since the vaccine's efficacy in activating an immune response is closely associated with the fully intact vaccine amount, we assessed the vaccine mRNA's quality and extent of degradation in the samples using ddPCR linkage duplex assay.[5]

Results

The vaccine mRNA was detected in the two placentas tested (Table) using quantitative ddPCR and ISH. The localization of the vaccine mRNA was mainly in the villus stroma (panels Ab and Ad), with a notably high signal in the decidua of patient 1 (panel Aa) compared to that of patient 2 (Panel Ac). Using WES, the Spike protein expression was detected in the placenta of patient # 2, but not in patient #1, as demonstrated in panel Aa. Furthermore, the vaccine mRNA was detected in the cord and maternal blood of patient #1 using ddPCR (Table). Unfortunately, no umbilical cord or maternal blood samples were available for analysis in patient #2. Finally, the integrity of the vaccine mRNA varied across different samples. In the placentas, 23% and 42% of the original integrity were retained in patients 1 and 2, respectively (Table 1). The vaccine mRNA

in the maternal blood showed a high integrity level of 85%; however, in the cord blood, it decreased to 13% of the original vaccine mRNA's integrity (panels Bc and Bd).

Conclusions:

Our findings suggest that the vaccine mRNA is not localized to the injection site and can spread systemically to the placenta and umbilical cord blood. The detection of the spike protein in the placental tissue indicates the bioactivity of the vaccine mRNA reaching the placenta. Notably, the vaccine mRNA was largely fragmented in the cord blood and, to a lesser extent, in the placenta. To our knowledge, these two cases demonstrate, for the first time, the ability of the COVID-19 vaccine mRNA to penetrate the fetal-placental barrier and reach the intrauterine environment.

Two previous human studies by the same research group investigated the presence of COVID vaccine mRNA in the placenta, but with different methodologies and results.[7,8] The first study, using qRT-PCR, failed to detect mRNA in maternal blood, cord blood, or placental tissue, possibly due to the long interval between vaccination and delivery and the use of a single primer set not fully aligned with the mRNA-1273 vaccine.[7] In their subsequent study to improve the sensitivity of the detection, an RNAscope-based ISH assay was used, which also did not detect the vaccine mRNA. However, the probe used targeted the SARS-CoV-2 S gene rather than the vaccine mRNA sequence.[8] This can lead to inaccurate results due to the mismatch between the probe and the target sequence. In our study, we adopted a more sensitive and robust approach. We used two primer sets covering ~1.5 kb of the full-length mRNA vaccine to enhance detection sensitivity. Furthermore, we utilized ddPCR for more precise quantification of the vaccine mRNA, offering superior accuracy and sensitivity over RT-qPCR. Lastly, our RNAscope-based ISH assay used a probe tailored explicitly for the vaccine mRNA, thus ensuring more reliable detection.

In this report, the placental concentration of the vaccine mRNA was higher in patient #1 (delivered 2 days after vaccination) than in patient #2 (delivered 10 days after vaccination). This observation is likely attributable to the short half-life of the vaccine mRNA, leading to rapid degradation by day 10 post-vaccination. Conversely, the expression of the spike protein in the placenta of patient #2, but not in patient #1, suggests that more than two days are required post-vaccination for the mRNA to reach the placenta and be translated into the spike protein, which is then expressed in the placental tissue. Notably, a significant amount of the vaccine mRNA in patient #1's maternal blood was also detected in the cord blood (Table 1, approximately one-third). However, the vaccine mRNA integrity was significantly reduced to 13%. While the vaccine mRNA in cord blood seems fragmented, suggesting limited bioactivity, further investigation is required to determine the minimum amount of mRNA required to elicit an immune response in the fetus. Although our findings are novel, they represent only two cases, and validation through subsequent research is needed. Furthermore, the specific mechanisms and contributing factors that facilitate the transplacental transport of vaccine mRNA need further exploration.

The evidence overwhelmingly supports the COVID-19 vaccine's effectiveness in mitigating the morbidity and mortality related to the COVID-19 disease in pregnant and non-pregnant individuals. The widespread acceptance and proven safety of mRNA vaccines during the COVID-19 pandemic have opened doors for other mRNA therapies. While gene therapy, particularly mRNA-based treatments, shows promise, research on its perinatal delivery is still emerging. Prenatal therapy can be advantageous, as it offers early disease intervention and reduced immunogenicity. In experiments with pregnant rats, LNPs successfully delivered various mRNAs, including one potentially useful for treating fetal anemia.[3] Although introducing mRNA to the fetus may pose potentially plausible risks, it may also have biologically plausible benefits. The potential of mRNA-based interventions in addressing maternal and fetal health issues is profound. Such

insights could substantially advance the crafting of safer and more effective mRNA-based therapies during pregnancy.

Data Availability Statement

Raw data for every experiment are available upon request. Upon justifiable request, the sharing of de-identified data should be approved by the board of an investigational ethics committee.

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Statement of Ethics

New York University institutional review board approval (approval numbers: i21-01616 and i18-01692) was obtained before initiating the study.

Author Contributions

NH and XL had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

XL, NH, BB, MH, EG, CD, and MH: conceived, designed, and performed the experiments, analyzed and interpreted data.

NH, MC and MH: collection of medical history

XL, NH, MH and MC: manuscript writing, table and figure preparation and final approval of the manuscript.

All authors: revision and final approval of the manuscript.

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FIGURE LEGEND

<u>Panel A</u> COVID-19 vaccine mRNA detection in the placenta by in situ hybridization

Demonstrates COVID-19 vaccine mRNA detected in paraffin-embedded placental tissue using "in situ hybridization (RNAscope™)." Panels Aa and Ab represent samples from patient 1, demonstrating positive signals in the decidua (panel Aa) and the villi (panel Ab) using RNAscope™ Probe- S-encoding-mRNA-1273-C1. Panel Ac and Ad represent samples from patient 2, demonstrating positive signals in the decidua (panel Ac) and the villi (panel Ad) using RNAscope® Probe - S-encoding-BNT-162b2-C1.

<u>Panel B</u> Placental Spike protein expression and the vaccine mRNA integrity.

Demonstrates the expression of S protein in the placenta and the integrity of vaccine mRNA in cord and maternal blood. Panel Ba shows the expression of S protein in tissue lysate of placental biopsies from patients 1 and 2, analyzed by automated capillary western blot (WES). Control: pre-pandemic placenta sample. S: Full-length S protein.

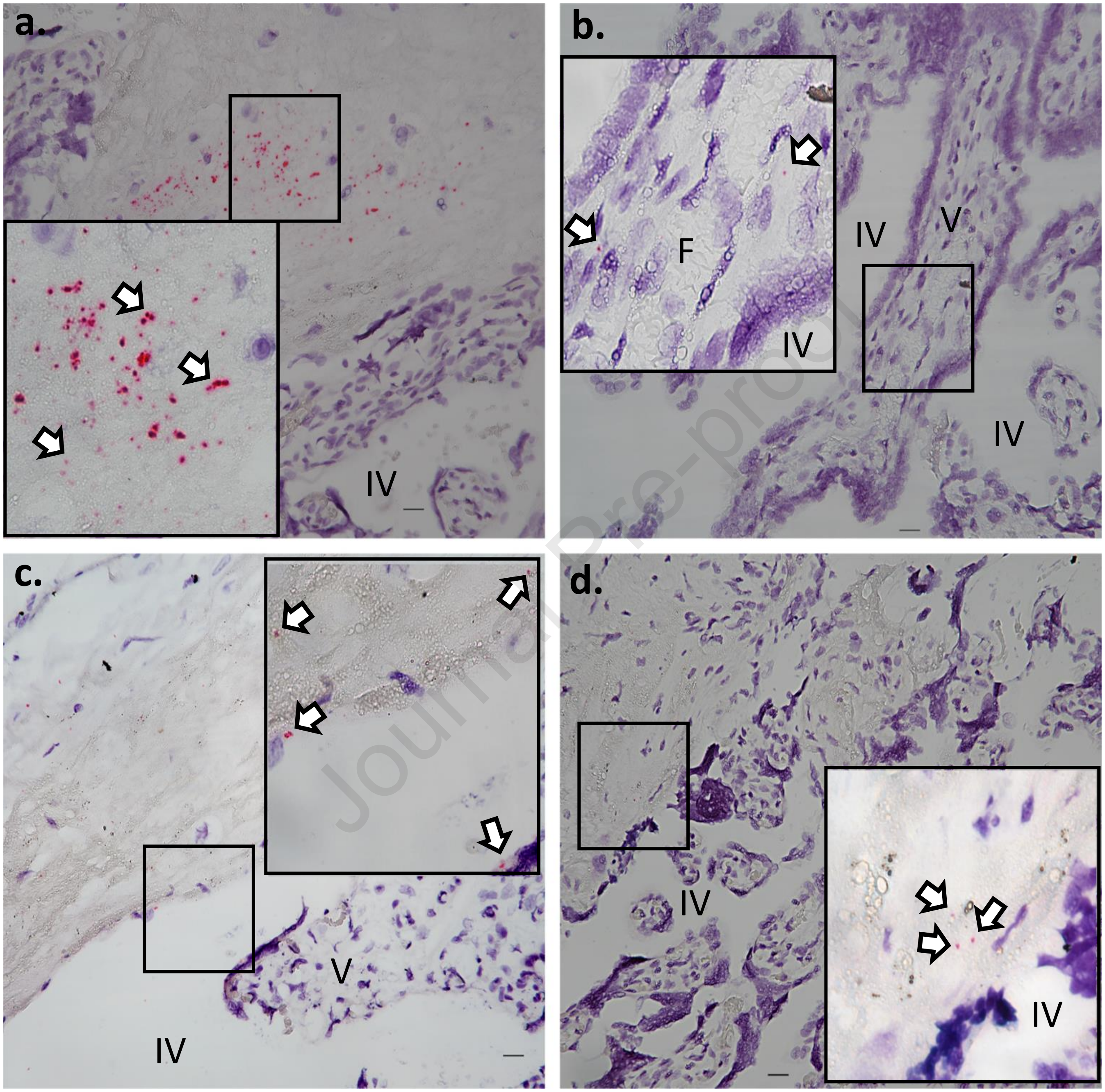
Circulating vaccine mRNA integrity was assayed in a duplex ddPCR assay in samples from patient 1 maternal blood (panel Bc, relative linkage 85%) and cord blood (panel Bd, relative linkage 13%). Panel Bb represents a blood sample of an unvaccinated subject showing no positive signal. Droplets emitting 2D signals were separated into four groups (Gray, double negative for mRNA1273-1 and mRNA1273-2; Blue, positive for mRNA1273-1, negative for mRNA1273-2; Green, positive for mRNA1273-2, negative for mRNA1273-1; Orange, double positive for both mRNA1273-1 and mRNA1273-2). The number of droplets in each single or double positive group was calculated by QX Manager Software, and the percent linkage of each sample was expressed as a percentage of linked molecules in relation to the total molecules detected normalized to the original vaccine stock solution.[5]

Table. Summary of vaccination history and vaccine mRNA and Spike protein detection.

	Patient 1	Patient 2
Gestational age	38 weeks +4 day	40 weeks +0 day
Birth type	Cesarean section	Vaginal delivery
COVID-19 disease history	One month before delivery	No COVID-19 history
Days between the last vaccination and delivery	2	10
Prior COVID-19 Vaccine history	Pfizer (3 doses) and one Moderna Booster	Pfizer (2 initial doses)
Last Vaccine type	Moderna Booster	Pfizer second dose
Vaccine mRNA detection in the placenta		
by ddPCR	5,033,000[a] (23%)[b]	1,387,000[a] (42%)[b]
by ISH	Detected	Detected
Spike Protein detection in the placenta		
by WES	Not Detected	Detected
Vaccine mRNA detection in maternal and cord blood		
Maternal blood (by ddPCR)	209,761[c] (85%)[b]	N/A
Cord blood (by ddPCR)	56,653[c] (13%)[b]	N/A

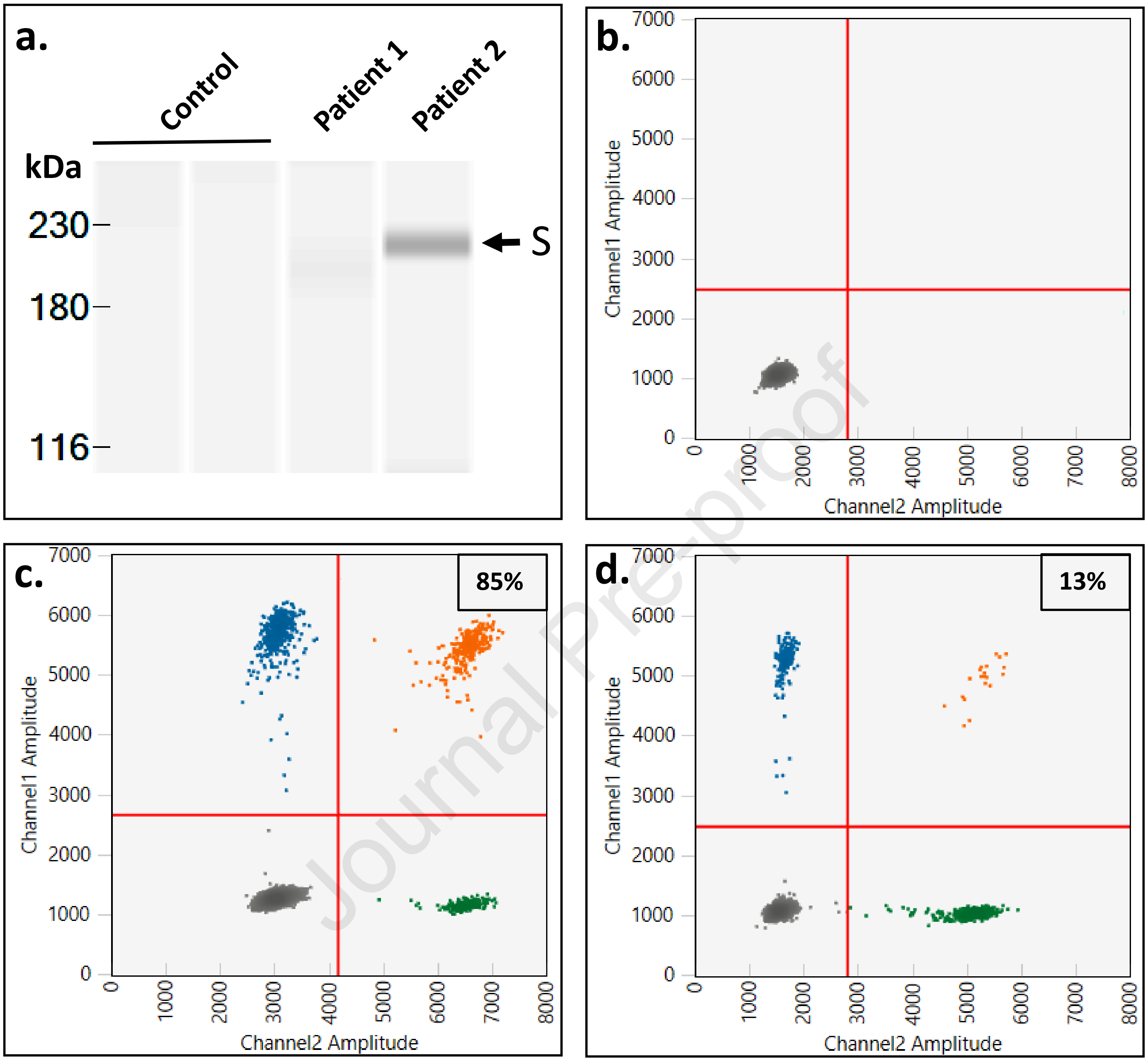
[a]mRNA copies per gram tissue. [b]Relative linkage. [c]Copies per mL blood.

Panel A



V, villi, IVS, intervillous space, FV, fetal vessel. Scale bar, 20 um.

Panel B


a.
Control
Patient 1
Patient 2
kDa
230
180
116
S
b.
c.
85%
d.
13%
Channel1 Amplitude
Channel2 Amplitude
0
1000
2000
3000
4000
5000
6000
7000
8000